

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: David P. Andrew, Brian A. Zabel and Paul D. Ponath

Divisional Application of:

Application No.: 09/266,464

Filed: March 11, 1999

For: METHODS OF IDENTIFYING AGENTS WHICH INHIBIT GPR-9-6

Date: 9.28.01

EXPRESS MAIL LABEL NO. EL551546111US

PRELIMINARY AMENDMENT

Box PATENT APPLICATION

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

Please amend the application as follows:

In the Specification

Replace the paragraph at page 4, lines 9 through 13 with the following paragraph:

The invention also relates to an isolated cell that produces an antibody or antigen-binding fragment of the present invention, including those which bind to mammalian GPR-9-6 and inhibit the binding of a ligand to the receptor. In a particular embodiment, the isolated cell is murine hybridoma 3C3 (also referred to as murine hybridoma LS129-3C3-E3-1) deposited under ATCC Accession No. HB-12653.

Replace the paragraph at page 6, lines 3 through 7 with the following paragraph:

Figures 2A-2B illustrate the specific binding of mAb 3C3 to GPR-9-6 transfectants. In Figure 2A, GPR-9-6/L1.2 transfectants were stained with mAb 3C3 (stippled profile), anti-CCR6 antibody (.....) or with a murine IgG2b mAb (----) (n=2). In Figure 2B, CCR6/L1.2 transfectants were stained with mAb 3C3 (.....), anti-CCR6 antibody (stippled profile) or with a murine IgG2b mAb (----) (n=2).

Replace the paragraph at page 6, lines 8 through 16 with the following paragraph:

Figures 3A-3I are a series of fluorescence plots which illustrate that GPR-9-6 is expressed on B lymphocytes and subsets of CD4 and CD8 lymphocytes. mAb 3C3 was used in two color studies on mononuclear cells along with anti-CD4 FITC (Figure 3A), anti-CD8 FITC (Figure 3B), anti-CD19 FITC (Figure 3C), anti-CD56 Cychrome (Figure 3D) and anti-CCR3 FITC (Figure 3E). For thymocytes (Figure 3F), two color studies were performed with mAb 3C3 and anti-TcR Cychrome. GPR-9-6 expression on monocytes (Figure 3G), eosinophils (Figure 3H) and neutrophils (Figure 3I) was evaluated in one color studies using isolated populations of these cells and mAb 3C3 (—) and IgG2b controls (----). Anti-CCR2, anti-CCR3 and anti-CXCR2 antibodies were used as positive controls for monocytes, eosinophils and neutrophils, respectively (stippled profiles) (n=3).

Replace the paragraph at page 6, lines 17 through 26 with the following paragraph:

Figures 4A-4H are plots illustrating that GPR-9-6 is not expressed on immature dendritic cells (IMDC), mature dendritic cells (MDC) or T_H1/T_H2 lymphocytes. Mature (—) and immature dendritic cells (stippled profile) were stained with anti-CCR5 (Figure 4A), anti-CD83 (Figure 4B), anti-CD86 (Figure 4C) or anti-GPR-9-6 (Figure 4D). Staining with IgG2b control on IMDCs (····) is also shown. Figure 4E shows staining of umbilical CD4 lymphocytes with anti-CXCR4 (stippled profile), anti-GPR-9-6 (—) and IgG2b (····). Figures 4F-4H show staining of T_H1 (stippled profiles) and T_H2 (—) lymphocytes with anti-CXCR3 (Figure 4F), anti- $\alpha 4\beta 7$ (Act1) (Figure 4G) or anti-GPR-9-6 (mAb 3C3) (Figure 4H) as indicated, with (····) representing staining with an IgG2b control on T_H1 lymphocytes (n=3).

Replace the paragraph at page 7, lines 8 through 16 with the following paragraph:

Figures 6A-6F are a series of fluorescence plots illustrating that GPR-9-6 is expressed on $\alpha 4\beta 7^{\text{high}}$ CLA^{-ve} CD4⁺ memory lymphocytes. Mononuclear cells were stained in three color experiments using anti-CD4 cychrome to gate on CD4 lymphocytes. The cells were also stained with anti-GPR-9-6 mAb 3C3 followed by F(ab')₂ anti-mouse IgG phycoerythrin to study GPR-9-6 expression on subsets defined with anti- αE (HML1, Beckman Coulter, Inc., Fullerton, CA) (Figure 6A), anti- $\beta 7$ (Fib504, PharMingen, San Diego, CA) (Figure 6B), anti-CD49d (HP2/1, PharMingen, San Diego, CA) (Figure 6C), anti-CLA (HECA 452, PharMingen, San Diego, CA) (Figure 6D), anti-CD45RO (UCLH1, PharMingen, San Diego, CA) (Figure 6E) and anti-CD62L (CD56)(PharMingen, San Diego, CA) (Figure 6F) (n=5).

Replace the paragraph at page 7, lines 17 through 26 with the following paragraph:

Figures 7A-7F are a series of fluorescence plots illustrating the expression of GPR-9-6 on CD4 lymphocytes in relation to other chemokine receptors. Mononuclear cells were stained in

three-color experiments using anti-CD4 cychrome to gate on CD4 lymphocytes. The cells were also stained with anti-GPR-9-6 mAb 3C3 followed by F(ab')₂ anti-mouse IgG coupled to phycoerythrin to study GPR-9-6 expression on subsets defined with anti-CCR2 (R&D Systems, Minneapolis, MN) (Figure 7A), anti-CCR5 (PharMingen, San Diego, CA) (Figure 7B), anti-CCR6 (R&D Systems, Minneapolis, MN) (Figure 7C), anti-CXCR3 (1C6, Leukosite, Inc., Cambridge, MA) (Figure 7D), anti-CXCR4 (PharMingen, San Diego, CA) (Figure 7E) and anti-CXCR5 (R&D Systems, Minneapolis, MN) (Figure 7F), all of which were coupled to phycoerythrin (n=2).

Replace the paragraph at page 8, lines 15 through 24 with the following paragraph:

Figures 10A-10F are a series of histograms illustrating that a subset of CD4 lymphocytes and thymocytes chemotax to TECK. CD4⁺ lymphocytes (Figure 10F), CD8⁺ lymphocytes (Figure 10B), CD56⁺ NK cells (Figure 10D) and CD14⁺ monocytes (Figure 10A) were isolated from mononuclear cells using the appropriate Miltenyi Beads. Neutrophils (Figure 10E) were isolated by dextran precipitation followed by Ficoll and eosinophils (Figure 10C) separated from neutrophils by depletion with anti-CD16 Miltenyi Beads. Uncoated 3 μ m Costar plates were used to assess chemotaxis with these leukocyte subsets, with the exception of eosinophils and neutrophils, for which ECV304 monolayers were grown over the inserts before the assay. In each case, TECK was tested in a dose response fashion between 1 nM and 220 nM. Chemokines known to act on the leukocyte subsets were used as positive controls (n=2).

Replace the paragraph bridging pages 14 and 15 with the following paragraph:

As described herein, an antibody designated "mAb 3C3" that binds human GPR-9-6 has been produced. mAb 3C3 can be produced by murine hybridoma 3C3, also referred to as murine hybridoma LS129-3C3-E3-1 which was deposited on March 4, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A., now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 01239, at the American Type Culture Collection, 10801

University Boulevard, Manassas, Virginia 20110-2209, U.S.A., under Accession No. HB-12653. In another embodiment, the anti-GPR-9-6 antibody of the invention is mAb 3C3 or an antigen-binding fragment thereof. In another embodiment, the binding of the antibody or antigen-binding fragment to mammalian (e.g., human) GPR-9-6 can be inhibited by mAb 3C3. Such inhibition can be the result of competition for the same or similar epitope, steric interference or due to a change in the conformation of GPR-9-6 that is induced upon antibody binding to the receptor. In still another embodiment, the antibody or antigen-binding fragment of the invention has the same or similar epitopic specificity as mAb 3C3. Antibodies with an epitopic specificity which is the same as or similar to that of mAb 3C3 can be identified by a variety of suitable methods. For example, an antibody with the same or similar epitopic specificity as mAb 3C3 can be identified based upon the ability to compete with mAb 3C3 for binding to mammalian GPR-9-6. In another example, the binding of mAb 3C3 and the binding of an antibody with the same or similar epitopic specificity to mammalian GPR-9-6 can be inhibited by a single peptide (e.g., natural peptide, synthetic peptide). The peptide can comprise nine to about fifty amino acids. Preferably, the peptide comprises nine to about twenty-six amino acids. In still another example, an antibody with the same or similar epitopic specificity as mAb 3C3 can be identified using chimeric receptors (see e.g., Rucker *et al.*, *Cell* 87:437-446 (1996)).

Replace the paragraph at page 15, lines 22 through 28 with the following paragraph:

The invention also relates to a bispecific antibody, or functional fragment thereof (e.g., F(ab')₂), which binds to a mammalian GPR-9-6 and at least one other antigen. In a particular embodiment, the bispecific antibody, or functional fragment thereof has the same or similar epitopic specificity as mAb 3C3 and at least one other antibody (see, e.g., U.S. Patent No. 5,141,736 (Iwasa *et al.*), U.S. Patent Nos. 4,444,878, 5,292,668, 5,523,210 (all to Paulus *et al.*) and U.S. Patent No. 5,496,549 (Yamazaki *et al.*)).

Replace the paragraph bridging pages 19 and 20 with the following paragraph:

In one embodiment, a functional variant of mammalian GPR-9-6 (e.g., a ligand binding variant) shares at least about 80% amino acid sequence similarity with said mammalian GPR-9-6, preferably at least about 90% amino acid sequence similarity, and more preferably at least about 95% amino acid sequence similarity with said mammalian GPR-9-6. In another embodiment, a functional fusion protein comprises a first moiety which shares at least about 85% sequence similarity with a mammalian GPR-9-6, preferably at least about 90% sequence similarity, and more preferably at least about 95% sequence similarity with a mammalian GPR-9-6 (e.g., a human GPR9-6 (e.g., SEQ ID NO:2)). In another embodiment, a functional mammalian GPR-9-6 protein or functional variant of a mammalian GPR-9-6 protein shares at least about 80% amino acid sequence similarity, preferably at least about 90% amino acid sequence similarity, and more preferably at least about 95% amino acid sequence similarity with a naturally occurring human GPR-9-6 (e.g., SEQ ID NO:2). Amino acid sequence similarity can be determined using a suitable sequence alignment algorithm, such as the LASERGENE system (sequence assembly and alignment software; DNASTAR, Inc., Madison, WI), using the Clustal method with the PAM 250 residue weight table, a gap penalty of 10, a gap length penalty of 10 and default parameters (pairwise alignment parameters: ktuple = 1, gap penalty = 3, window = 4 and diagonals saved = 5). In another embodiment, a functional variant is encoded by a nucleic acid sequence which is different from the naturally-occurring nucleic acid sequence, but which, due to the degeneracy of the genetic code, encodes mammalian GPR-9-6 or a portion thereof.

Replace the paragraph bridging pages 20 and 21 with the following paragraph:

A composition comprising a mammalian GPR-9-6 or functional variant thereof can be used in a binding assay to detect and/or identify agents that can bind to the receptor. Compositions suitable for use in a binding assay include, for example, cells which naturally express a mammalian GPR-9-6 or functional variant thereof (e.g., thymocytes, GPR-9-6⁺ CLA^{-ve} $\alpha 4\beta 7^{hi}$ CD4⁺ memory lymphocytes, cell lines (e.g., MOLT-4 (ATCC Accession No. CRL-1582),

MOLT-13 (M. Brenner, Brigham and Women's Hospital, Boston, MA)) and recombinant cells comprising an exogenous nucleic acid sequence which encodes a mammalian GPR-9-6 or functional variant thereof. Compositions suitable for use in a binding assay also include, membrane preparations which comprise a mammalian GPR-9-6 or functional variant thereof. Such membrane preparations can contain natural (e.g., plasma membrane) or synthetic membranes. Preferably, the membrane preparation is a membrane fraction of a cell that expresses a mammalian GPR-9-6 or a functional variant thereof.

Replace the paragraph at page 25, lines 3 through 15 with the following paragraph:

An agent which binds to a mammalian GPR-9-6 can also be assessed by monitoring cellular responses induced by active receptor, using suitable cells which express a mammalian GPR-9-6 or a functional variant thereof. For instance, exocytosis (e.g., degranulation of cells leading to release of one or more enzymes or other granule components, such as esterases (e.g., serine esterases), perforin, and/or granzymes), inflammatory mediator release (such as release of bioactive lipids such as leukotrienes (e.g., leukotriene C₄)), and respiratory burst, can be monitored by methods known in the art or other suitable methods (see e.g., Taub, D.D. *et al.*, *J. Immunol.*, 155: 3877-3888 (1995), regarding assays for release of granule-derived serine esterases; Loetscher *et al.*, *J. Immunol.*, 156: 322-327 (1996), regarding assays for enzyme and granzyme release; Rot, A. *et al.*, *J. Exp. Med.*, 176: 1489-1495 (1992) regarding respiratory burst; Bischoff, S.C. *et al.*, *Eur. J. Immunol.*, 23: 761-767 (1993) and Baggolini, M. and C.A. Dahinden, *Immunology Today*, 15: 127-133 (1994)).

Replace the paragraph bridging pages 27 and 28 with the following paragraph:

In vivo models of inflammation are available which can be used to assess the efficacy of antibodies and antigen-binding fragments of the invention as well as agents identified by the methods described herein as *in vivo* as therapeutics. For example, leukocyte infiltration upon intradermal injection of a chemokine and an antibody or fragment thereof reactive with

mammalian GPR-9-6 into a suitable animal, such as rabbit, mouse, rat, guinea pig or primate (e.g., rhesus macaque) can be monitored (see e.g., Van Damme, J. *et al.*, *J. Exp. Med.*, 176: 59-65 (1992); Zachariae, C.O.C. *et al.*, *J. Exp. Med.* 171: 2177-2182 (1990); Jose, P.J. *et al.*, *J. Exp. Med.* 179: 881-887 (1994)). In one embodiment, skin biopsies are assessed histologically for infiltration of leukocytes (e.g., GPR-9-6⁺ T cells). In another embodiment, labeled cells (e.g., stably transfected cells expressing a mammalian GPR-9-6, labeled with ¹¹¹In for example) capable of chemotaxis and extravasation are administered to the animal. For example, an antibody or agent to be assessed which binds a mammalian GPR-9-6 can be administered, either before, simultaneously with or after a GPR-9-6 ligand or agonist (e.g., TECK) is administered to the test animal. A decrease of the extent of infiltration in the presence of antibody or agent as compared with the extent of infiltration in the absence of said antibody or agent is indicative of inhibition.

Replace the paragraph bridging pages 41 and 42 with the following paragraph:

Plasmid DNA was isolated using QIAGEN-tips as recommended by the manufacturer (QIAGEN Inc., Chatsworth, CA). DNA ligations, restriction endonuclease digestions, and gel electrophoresis were performed as described previously (Sambrook, J., *et al.*, *Molecular Cloning: A Laboratory Manual 2nd ed.*, Cold Spring Harbor Laboratory Press, (Cold Spring Harbor, NY) (1989)). DNA purification through agarose gel extraction was performed using the QIAEXII Gel Extraction Kit as recommended by the manufacturer (QIAGEN Inc., Chatsworth, CA). Plasmid DNA was introduced into *E. coli* by chemical transformation (GIBCO, Inc.). Enzymes were purchased from New England Biolabs, Inc. (Beverly, MA), GIBCO Bethesda Research Laboratories, Inc. (Gaithersburg, MD), or from Boehringer Mannheim, Inc. (Germany). RNA was isolated from frozen tissues or cells using either the standard guanidinium isothiocyanate method (Sambrook, J., *et al.*, *Molecular Cloning: A Laboratory Manual 2nd ed.*, Cold Spring Harbor Laboratory Press, (Cold Spring Harbor, NY) (1989)) or the RNeasy kit as recommended (QIAGEN Inc., Chatsworth, CA). DNA sequencing was performed by Sequi-Net (Colorado State University) using the FS DyeDeoxy Terminator cycle sequencing kit and a

model 377 DNA sequencer (Perkin Elmer Applied Biosystems, Foster City, CA). Sequences were analyzed using SeqMan (DNASTAR, Inc., Madison, WI).

Replace the paragraph bridging pages 45 and 46 with the following paragraph:

In initial two color studies of peripheral blood, GPR-9-6 was found to be expressed on a small subset (2-4%) of CD4 lymphocytes as well as on a very small subset of CD8 lymphocytes, while B lymphocytes expressed low and heterogeneous levels of GPR-9-6 (Figures 3A-3C). Monocytes, basophils, eosinophils, neutrophils and NK cells did not express GPR-9-6 under the conditions used (Figures 3D-3I). GPR-9-6 was expressed on a large subset of thymocytes expressing all levels of TcR, although a small subset of $TcR^{high}GPR-9-6^{+ve}$ thymocytes was evident. In three-color experiments, GPR-9-6 was found on the majority of CD4, CD8 and $CD4^{+ve}CD8^{+ve}$ thymocytes and on approximately 50% of immature $CD4^{-ve}CD8^{-ve}$ thymocytes (data not shown). No expression of GPR-9-6 was seen on either immature or mature dendritic cells (Figure 4D). However, as expected, immature dendritic cells expressed CCR5, which was down-regulated on LPS activation, while CD83 and CD86 were up-regulated (Figures 4A-4C). In examining a large panel of cell lines GPR-9-6 was found on several T cell lines (Table 1). Umbilical CD4+ lymphocytes did not express GPR-9-6 (Figure 4E) and chronic activation of these cells in the presence of IL-12 or IL-4 to generate T_H1 or T_H2 lymphocytes failed to induce the expression of GPR-9-6 (Figure 4H). However, as expected, CXCR3 were clearly up-regulated on T_H1 lymphocytes (Figure 4F), while $\alpha 4\beta 7$, an integrin utilized in lymphocyte trafficking to mucosal sites, was up-regulated on both T_H1 and T_H2 lymphocytes (Figure 4G).

Replace the paragraph at page 46, lines 5 through 10 with the following paragraph:

Expression of GPR-9-6 on CD4 lymphocytes and B lymphocytes was measured over time, and was found to be relatively constant (Figure 5A). However, activation of T lymphocytes with anti-CD3 mAb resulted in transient down-regulation of GPR-9-6 over 2 days, with

expression recovering after 10 days of culture in IL-2 (Figure 5B). Chemokine receptors CCR6 and CCR5 showed similar changes in expression upon T lymphocyte activation (Figure 5C).

Replace the paragraph at page 46, lines 14 through 24 with the following paragraph:

The small subset of CD4 lymphocytes that express GPR-9-6 were examined in more detail by three-color staining (Figures 6A-6F). The CD4 lymphocytes that express GPR-9-6 were mainly of memory phenotype, and those cells that expressed the highest levels of GPR-9-6 were all of memory phenotype. Interestingly, memory CLA^{+ve} CD4 lymphocytes, which traffic to skin, did not express GPR-9-6. In contrast, a subset of memory $\alpha 4\beta 7^{\text{high}}$ CD4 lymphocytes, which traffic to mucosal sites, clearly expressed GPR-9-6. The subset of memory CD4 lymphocytes defined by expression of $\alpha E\beta 7$ were also clearly subdivided into GPR-9-6 positive and negative subsets. GPR-9-6^{high} CD4 lymphocytes did not express CD62L, a homing receptor which is involved in trafficking to peripheral lymph nodes, while a small subset of GPR-9-6^{dull}CD62L^{+ve} lymphocytes was evident.

Replace the paragraph bridging pages 46 and 47 with the following paragraph:

GPR-9-6^{+ve} CD4 lymphocytes were also examined for co-expression of other chemokine receptors known to be expressed on CD4 lymphocytes (Figures 7A-7F). While GPR-9-6 was clearly found on both positive and negative subsets of CCR5, CCR6, CXCR3 and CXCR5, CD4 lymphocyte expression of CCR2 and GPR-9-6 was mutually exclusive.

Replace the paragraph at page 49, lines 1 through 10 with the following paragraph:

Leukocyte subsets were also tested (Figures 10A-10F) to determine if they chemotaxed to TECK. As observed in the mouse, neutrophils, monocytes, eosinophils, CD8 and NK cells did not chemotax to TECK, but did chemotax to other chemokines. However, TECK was chemotactic for a minor subset of CD4 lymphocytes. As murine TECK induces thymocyte

chemotaxis, chemotaxis of human thymocytes to TECK and SDF1 α , both of which mediate thymocyte chemotaxis (data not shown) was examined. Anti-GPR-9-6 mAb 3C3 blocked thymocyte and CD4 lymphocyte chemotaxis to TECK. The anti-GPR-9-6 mAb 3C3 had no effect on TARC-induced chemotaxis of CD4 lymphocytes, indicating that the effect is specific (Figures 11A-11C). These results indicate that GPR-9-6 is the major physiological receptor for TECK.

Replace the paragraph at page 50, lines 7 through 20 with the following paragraph:

Several different adhesion molecules are involved in trafficking of lymphocyte subsets to distinct physiologic location, such as peripheral lymph node (Gallatin, W.M., *et al.*, *Nature*, 304:30-34 (1983)), Peyer's Patches (Hamman, A., *et al.*, *J. Immunol.*, 152:3282-3292 (1994); Andrew, D.P., *et al.*, *Eur. J. Immunol.*, 26:897-905 (1996)) and inflammatory sites (Frenette, P.S., *et al.*, *Cell*, 84:563-574 (1996); Tietz, W.Y., *et al.*, *J. Immunol.*, 161(2):963-970 (1998); Picker, L.J., *et al.*, *J. Immunol.*, 145:3247-3255 (1990)). It is thought that specific chemokine receptors expressed on these lymphocyte subsets may interact with chemokines expressed in the areas mediating leukocyte activation, arrest, and transendothelial migration. It is therefore possible that CD4 subsets defined by the expression of certain adhesion molecules, may also express known, orphan or as yet undiscovered chemokine receptors that are important for trafficking of the lymphocytes into these sites. The work described herein relates to one such chemokine receptor that may be involved in the selective trafficking of memory CD4 and CD8 lymphocyte subsets to mucosal sites.

Replace the paragraph bridging pages 52 and 53 with the following paragraph:

Out of all the chemokines tested only TECK (Vicari, A.P., *et al.*, *Immunity*, 7(2):291-301 (1997)) acted as a chemoattractant for GPR-9-6/L1.2 transfectants, with 150 nM resulting in optimal chemotaxis. This falls into the range of 1nM-1 μ M for which other leukocyte chemokines are active. However, as we are using TECK that was generated by peptide synthesis,

we cannot be sure that either post-translational modifications or further cleavage of TECK by factors outside the cell *in vivo* do not generate more active fragments, as is the case for CKB8 (Macphee, C.H., *et al.*, *J. Immunol.* 161:6273-6279 (1998)). TECK did not act as a chemoattractant for CCR1, CCR2, CCR4, CCR5, CCR6, CCR7, CCR9 and CXCR1, CXCR2, CXCR3, CXCR4 and CXCR5 L1.2 transfectants. However, some weak activity of TECK on CCR3/L1.2 transfectants which was approximately 20% of the chemotactic activity observed with eotaxin-1 was detected. This activity was blocked by anti-CCR3 mAbs, though TECK did not act as a chemoattractant for eosinophils. Therefore, TECK is probably not a physiological chemokine for the CCR3 receptor. This result is not unprecedented, as in previous studies MIP-1 α has been shown to act as a chemoattractant for CCR4/HEK293 transfectants (Power, C.A., *et al.*, *J. Biol. Chem.*, 270:19495-19500 (1995)), but not CCR4/L1.2 transfectants (Imai, T.M., *et al.*, *J. Biol. Chem.*, 272:15036-15042 (1997)). In further experiments, only the T cell lines that express GPR-9-6 were found to chemotax to TECK, while among primary cells TECK was chemotactic for only a small subset of CD4 lymphocytes. Presumably, these cells represent the small subset of CD4 lymphocytes that express GPR-9-6, as the chemotaxis was blocked by anti-GPR-9-6 mAb 3C3. Additionally, only $\alpha 4\beta 7^{+ve}$ memory CD4 and CD8 lymphocytes chemotax to TECK, which would be the subset predicted to express GPR-9-6. TECK was originally described as a chemokine produced by thymic dendritic cell, whose expression is restricted to thymus and small intestine (Vicari, A.P., *et al.*, *Immunity*, 7(2):291-301 (1997)). Our Northern data confirms this observation and shows that the receptor for TECK, GPR-9-6, is also expressed at these sites. The expression of both chemokine receptor GPR-9-6 and its ligand TECK in small intestine and thymus predict a role for GPR-9-6 and TECK in T cell development and mucosal immunology.

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i-xii).

In the Claims

Please cancel Claims 1-24 and 29-49. Claims 25 and 28 have been amended and are presented below in amended form, and Claims 50-96 have been added. In accordance with 37 C.F.R. § 1.121(c)(1)(ii), amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (page xii).

25. (Amended) A method of detecting or identifying an inhibitor of a mammalian GPR-9-6 receptor comprising:
 - a) combining an agent to be tested, a ligand or promoter of mammalian GPR-9-6 and a cell expressing mammalian GPR-9-6 under conditions suitable for detecting a ligand- or promoter-induced response; and
 - b) determining the ability of the test agent to inhibit said ligand- or promoter-induced response,
wherein inhibition of said ligand- or promoter-induced response by the agent is indicative that the agent is an inhibitor.
28. (Amended) The method of Claim 25 wherein said ligand- or promoter-induced response is chemotaxis.
50. (New) The method of Claim 25 wherein said ligand- or promoter-induced response is Ca^{2+} flux.
51. (New) The method of Claim 25 wherein said cell is a recombinant cell.
52. (New) The method of Claim 25 wherein said cell is a cell line.
53. (New) The method of Claim 52 wherein said cell line is selected from the group consisting of MOLT-4 and MOLT-13.

54. (New) The method of Claim 25 wherein said mammalian GPR-9-6 is a human GPR-9-6.
55. (New) The method of Claim 25 wherein said mammalian GPR-9-6 comprises the amino acid sequence of SEQ ID NO:2.
56. (New) The method of Claim 25 wherein said test agent is an organic compound.
57. (New) The method of Claim 25 wherein said test agent is an antibody or antigen-binding fragment of an antibody.
58. (New) The method of Claim 25 wherein said test agent is a peptide.
59. (New) The method of Claim 25 wherein said test agent is a nucleic acid.
60. (New) A method of detecting or identifying an inhibitor of a mammalian GPR-9-6 receptor comprising:
 - a) combining an agent to be tested, a ligand or promoter of mammalian GPR-9-6 and a cell expressing mammalian GPR-9-6 or functional variant thereof under conditions suitable for detecting a ligand- or promoter-induced response, wherein said functional variant binds TECK and mediates TECK-induced signaling or a TECK-induced cellular response; and
 - b) determining the ability of the test agent to inhibit said ligand- or promoter-induced response, wherein inhibition of said ligand- or promoter-induced response by the agent is indicative that the agent is an inhibitor.
61. (New) The method of Claim 60 wherein said ligand or promoter of mammalian GPR-9-6 is TECK.

62. (New) The method of Claim 60 wherein said cell is a recombinant cell.
63. (New) The method of Claim 60 wherein said cell is a cell line.
64. (New) The method of Claim 63 wherein said cell line is selected from the group consisting of MOLT-4 and MOLT-13.
65. (New) The method of Claim 60 wherein said ligand- or promoter-induced response is chemotaxis.
66. (New) The method of Claim 60 wherein said ligand- or promoter-induced response is Ca^{2+} flux.
67. (New) The method of Claim 60 wherein said mammalian GPR-9-6 or functional variant thereof is a human GPR-9-6 or functional variant thereof.
68. (New) The method of Claim 60 wherein said mammalian GPR-9-6 or functional variant is a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or a functional variant of said polypeptide.
69. (New) The method of Claim 60 wherein said test agent is an organic compound.
70. (New) The method of Claim 60 wherein said test agent is an antibody or antigen-binding fragment of an antibody.
71. (New) The method of Claim 60 wherein said test agent is a peptide.
72. (New) The method of Claim 60 wherein said test agent is a nucleic acid.

73. (New) A method of detecting or identifying an inhibitor of a mammalian GPR-9-6 receptor comprising:

- a) combining an agent to be tested, a ligand or promoter of mammalian GPR-9-6 and a cell expressing a protein comprising GPR-9-6 under conditions suitable for detecting a ligand- or promoter-induced response, wherein said GPR-9-6 binds TECK and comprises an amino acid sequence that is at least about 90% similar to the amino acid sequence of SEQ ID NO:2; and
- b) determining the ability of the test agent to inhibit said ligand- or promoter-induced response,
wherein inhibition of said ligand- or promoter-induced response by the agent is indicative that the agent is an inhibitor.

74. (New) The method of Claim 73 wherein said ligand or promoter of mammalian GPR-9-6 is TECK.

75. (New) The method of Claim 73 wherein said cell is a recombinant cell.

76. (New) The method of Claim 73 wherein said cell is a cell line.

77. (New) The method of Claim 76 wherein said cell line is selected from the group consisting of MOLT-4 and MOLT-13.

78. (New) The method of Claim 73 wherein said ligand- or promoter-induced response is chemotaxis.

79. (New) The method of Claim 73 wherein said ligand- or promoter-induced response is Ca^{2+} flux.

80. (New) The method of Claim 73 wherein said GPR-9-6 is a human GPR-9-6.

81. (New) The method of Claim 73 wherein said GPR-9-6 comprises the amino acid sequence of SEQ ID NO:2.
82. (New) The method of Claim 73 wherein said test agent is an organic compound.
83. (New) The method of Claim 73 wherein said test agent is an antibody or antigen-binding fragment of an antibody.
84. (New) The method of Claim 73 wherein said test agent is a peptide.
85. (New) The method of Claim 73 wherein said test agent is a nucleic acid.
86. (New) A method of detecting or identifying an inhibitor of a human GPR-9-6 receptor comprising:
 - a) combining an agent to be tested, TECK and a cell expressing a protein comprising human GPR-9-6 under conditions suitable for detecting a ligand- or promoter-induced response; and
 - b) determining the ability of the test agent to inhibit said response, wherein inhibition of said ligand- or promoter-induced response by the agent is indicative that the agent is an inhibitor.
87. (New) The method of Claim 86 wherein said cell is a recombinant cell.
88. (New) The method of Claim 86 wherein said cell is a cell line.
89. (New) The method of Claim 88 wherein said cell line is selected from the group consisting of MOLT-4 and MOLT-13.

90. (New) The method of Claim 86 wherein said ligand- or promoter-induced response is chemotaxis.
91. (New) The method of Claim 86 wherein said ligand- or promoter-induced response is Ca^{2+} flux.
92. (New) The method of Claim 86 wherein said human GPR-9-6 comprises the amino acid sequence of SEQ ID NO:2.
93. (New) The method of Claim 86 wherein said test agent is an organic compound.
94. (New) The method of Claim 86 wherein said test agent is an antibody or antigen-binding fragment of an antibody.
95. (New) The method of Claim 86 wherein said test agent is a peptide.
96. (New) The method of Claim 86 wherein said test agent is a nucleic acid.

REMARKS

The subject application is a divisional of U.S. Application No. 09/266,464, filed March 11, 1999. Original Claims 1-24 and 29-49 have been cancelled and new Claims 50-96 have been added. Claims 25-28 and new Claims 50-96 are directed to the invention of Group IV, a method of detecting an inhibitor of GPR-9-6, as defined in the Restriction Requirement dated October 4, 2000 (Paper No. 9), which issued in the parent application (U.S. Application No. 09/266,464).

Amendments to the Specification

In accordance with 37 C.F.R. § 1.121(b)(1)(i and ii), this Amendment includes instructions to replace certain paragraphs of the specification with replacement paragraphs.

The subject divisional application is being filed with Formal Drawings. In the Formal

Drawings, Figures 3, 6, 7 and 10 as originally filed in the parent application (application number 09/266,464) are re-labeled as FIGS. 3A-3I, 6A-6F, 7A-7F and 10A-10F, respectively. In addition, solid features of these drawings are now stippled. Accordingly, the specification has been amended as follows:

The paragraph at page 6, lines 3 through 7 has been amended to recite “stippled profile.”

The paragraph at page 6, lines 8 through 16 has been amended to refer to Figures 3A-3I, and to recite “stippled profile.”

The paragraph at page 6, lines 17 through 26 has been amended to recite “stippled profile.”

The paragraph at page 7, lines 8 through 16 has been amended to refer to Figures 6A-6F.

The paragraph at page 7, lines 17 through 26 has been amended to refer to Figures 7A-7F.

The paragraph at page 8, lines 15 through 24 has been amended to refer to Figures 10A-10F.

The paragraph bridging pages 45 and 46 has been amended to delete “Figure 3” and to recite “Figures 3A-3C” at line 3 of the paragraph and to recite “Figures 3D-3I” at line 5 of the paragraph.

The paragraph at page 46, lines 14 through 24 has been amended to delete “Figure 6” and insert therefor “Figures 6A-6F.”

The paragraph bridging pages 46 and 47 has been amended to delete “Figure 7” and insert therefor “Figures 7A-7F.”

The paragraph at page 49, lines 1 through 10 has been amended to delete “Figure 10” and insert therefor “Figures 10A-10F.”

The specification has also been amended to correct certain informalities. The replacement paragraphs include the following amendments correcting informalities:

At page 4, line 13 and at page 15, line 13 the blank line has been deleted and the ATCC Accession Number for murine hybridoma 3C3 (HB-12653) has been inserted therefor.

At page 6, line 3, the word “bind” has been deleted, and at line 4, “Figure 2B” has been deleted and “Figure 2A” has been inserted therefor.

At page 15, line 1, "now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 01239" has been inserted after "U.S.A.," and at line 3, "-2209" has been inserted.

At page 15, line 23 and line 25, the word "at" has been inserted after the word "and".

At page 20, line 12, "Lasergene" has been deleted and "LASERGENE" has been inserted therefor, and the phrase "sequence assembly and alignment software;" has been inserted immediately before "DNASTAR".

At page 20, line 25, "Womans" has been deleted and "Women's" has been inserted therefor.

At page 25, line 14, "Baggliolini" has been deleted and "Baggiolini" has been inserted therefor.

At page 27, line 18, the word "of" has been inserted after the word "efficacy".

At page 41, line 17, the word "the" has been inserted after the word "by".

At page 46, line 9, the word "shown" has been deleted and the word "showed" has been inserted therefor.

At page 50, line 8, "Gallitin" has been deleted and "Gallatin" inserted therefor, at line 9, "Peyers" has been deleted and "Peyer's" inserted therefor, and at line 19, the word "of" has been inserted after the word "trafficking".

At page 52, line 24, the word "not" has been inserted after the word "but".

Amendments to the Claims

Claims 1-24 and 29-49 have been cancelled. Claims 25 and 28 have been amended and new Claims 50-96 have been added. Claims 25-28 and 50-96 are pending.

Claim 25 has been amended to recite "mammalian GPR-9-6," "said ligand- or promoter-induced response," and "test agent."

Claim 28 has been amended to recite "said ligand- or promoter-induced response is chemotaxis."

Support of new Claims 50-96 is found throughout the specification.

Support for Claims 50, 65, 66, 78, 79, 90 and 91 is found, for example, at page 23, line 20 *et seq.*

Support for Claims 51-53, 62-64, 75-77 and 87-89 is found, for example, at page 20, lines 21-27 and at page 47, lines 4-21.

Support for Claims 54, 55, 67, 68, 73, 80, 81, 86 and 92 is found, for example, at page 19, line 27 through page 20, line 18, and Figure 15. A sequence alignment algorithm and parameters suitable for determining if an amino acid sequence is at least about 90% similar to the amino acid sequence of SEQ ID NO:2 are disclosed at page 20, lines 10-15.

Support for Claims 56-59, 69-72, 82-85 and 93-96 is found, for example, at page 23, lines 3-18.

Support for the recitation “wherein said ligand-binding variant binds TECK and mediates TECK-induced signaling or TECK-induced cellular response,” in Claim 60 is found, for example, in the paragraph bridging pages 18 and 19.

Support for the ligand or promoter of GPR-9-6 being TECK is found throughout the application, for example, at page 11, lines 2-4.

The amendments to the specification and claims are supported by the application as filed. Therefore, this Preliminary Amendment adds no new matter.

Biological Deposit

The application refers to murine hybridoma 3C3 that was deposited at the American Type Culture Collection (ATCC) on March 4, 1999, under the provisions of the Budapest Treaty. A copy of the ATCC Budapest Treaty Deposit Receipt and Viability Statement for murine hybridoma 3C3 is provided herewith.

Information Disclosure Statement

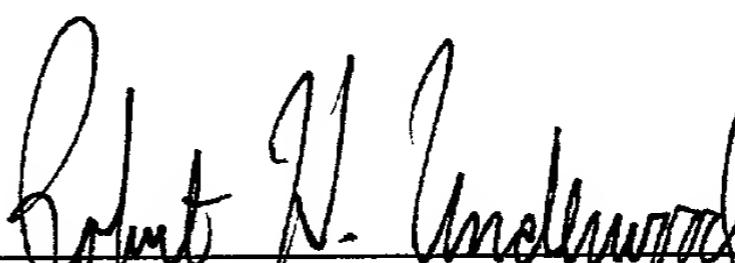
An Information Disclosure Statement (IDS) is being filed concurrently herewith. Acknowledgment of consideration of the information provided therein is request in the next Office Communication.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978)341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By 
Robert H. Underwood
Registration No. 45,170
Telephone (978) 341-0036
Facsimile (978) 341-0136

Lexington, Massachusetts 02421-4799

Dated: *September 28, 2001*

MARKED UP VERSION OF AMENDMENTSSpecification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

The paragraph at page 4, lines 9 through 13 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

The invention also relates to an isolated cell that produces an antibody or antigen-binding fragment of the present invention, including those which bind to mammalian GPR-9-6 and inhibit the binding of a ligand to the receptor. In a particular embodiment, the isolated cell is murine hybridoma 3C3 (also referred to as murine hybridoma LS129-3C3-E3-1) deposited under ATCC Accession No. [] HB-12653.

The paragraph at page 6, lines 3 through 7 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Figures 2A-2B illustrate the specific binding of mAb 3C3 [bind] to GPR-9-6 transfectants. In Figure [2B] 2A, GPR-9-6/L1.2 transfectants were stained with mAb 3C3 ([solid] stippled profile), anti-CCR6 antibody (.....) or with a murine IgG2b mAb (----) (n=2). In Figure 2B, CCR6/L1.2 transfectants were stained with mAb 3C3 (.....), anti-CCR6 antibody ([solid] stippled profile) or with a murine IgG2b mAb (----) (n=2).

The paragraph at page 6, lines 8 through 16 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

[Figure 3 is] Figures 3A-3I are a series of fluorescence plots which illustrate that GPR-9-6 is expressed on B lymphocytes and subsets of CD4 and CD8 lymphocytes. mAb 3C3 was used in two color studies on mononuclear cells along with anti-CD4 FITC (Figure 3A), anti-CD8 FITC (Figure 3B), anti-CD19 FITC (Figure 3C), anti-CD56 Cychrome (Figure 3D) and anti-CCR3 FITC (Figure

3E). For thymocytes (Figure 3F), two color studies were performed with mAb 3C3 and anti-TcR Cychrome. GPR-9-6 expression on monocytes (Figure 3G), eosinophils (Figure 3H) and neutrophils (Figure 3I) was evaluated in one color studies using isolated populations of these cells and mAb 3C3 (—) and IgG2b controls (----). Anti-CCR2, anti-CCR3 and anti-CXCR2 antibodies were used as positive controls for monocytes, eosinophils and neutrophils, respectively ([solid] stippled profiles) (n=3).

The paragraph at page 6, lines 17 through 26 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Figures 4A-4H are plots illustrating that GPR-9-6 is not expressed on immature dendritic cells (IMDC), mature dendritic cells (MDC) or T_H1/T_H2 lymphocytes. Mature (—) and immature dendritic cells ([solid] stippled profile) were stained with anti-CCR5 (Figure 4A), anti-CD83 (Figure 4B), anti-CD86 (Figure 4C) or anti-GPR-9-6 (Figure 4D). Staining with IgG2b control on IMDCs (.....) is also shown. Figure 4E shows staining of umbilical CD4 lymphocytes with anti-CXCR4 ([solid] stippled profile), anti-GPR-9-6 (—) and IgG2b (.....). Figures 4F-4H show staining of T_H1 ([solid] stippled profiles) and T_H2 (—) lymphocytes with anti-CXCR3 (Figure 4F), anti- $\alpha 4\beta 7$ (Act1) (Figure 4G) or anti-GPR-9-6 (mAb 3C3) (Figure 4H) as indicated, with (....) representing staining with an IgG2b control on T_H1 lymphocytes (n=3).

The paragraph at page 7, lines 8 through 16 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

[Figure 6 is] Figures 6A-6F are a series of fluorescence plots illustrating that GPR-9-6 is expressed on $\alpha 4\beta 7^{\text{high}}$ CLA^{-ve} CD4⁺ memory lymphocytes. Mononuclear cells were stained in three color experiments using anti-CD4 cychrome to gate on CD4 lymphocytes. The cells were also stained with anti-GPR-9-6 mAb 3C3 followed by F(ab')₂ anti-mouse IgG phycoerythrin to study GPR-9-6 expression on subsets defined with anti- αE (HML1, Beckman Coulter, Inc., Fullerton, CA) (Figure 6A), anti- $\beta 7$ (Fib504, PharMingen, San Diego, CA) (Figure 6B), anti-CD49d (HP2/1,

PharMingen, San Diego, CA) (Figure 6C), anti-CLA (HECA 452, PharMingen, San Diego, CA) (Figure 6D), anti-CD45RO (UCLH1, PharMingen, San Diego, CA) (Figure 6E) and anti-CD62L (CD56)(PharMingen, San Diego, CA) (Figure 6F) (n=5).

The paragraph at page 7, lines 17 through 26 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

[Figure 7 is] Figures 7A-7F are a series of fluorescence plots illustrating the expression of GPR-9-6 on CD4 lymphocytes in relation to other chemokine receptors. Mononuclear cells were stained in three-color experiments using anti-CD4 cychrome to gate on CD4 lymphocytes. The cells were also stained with anti-GPR-9-6 mAb 3C3 followed by F(ab')₂ anti-mouse IgG coupled to phycoerythrin to study GPR-9-6 expression on subsets defined with anti-CCR2 (R&D Systems, Minneapolis, MN) (Figure 7A), anti-CCR5 (PharMingen, San Diego, CA) (Figure 7B), anti-CCR6 (R&D Systems, Minneapolis, MN) (Figure 7C), anti-CXCR3 (1C6, Leukosite, Inc., Cambridge, MA) (Figure 7D), anti-CXCR4 (PharMingen, San Diego, CA) (Figure 7E) and anti-CXCR5 (R&D Systems, Minneapolis, MN) (Figure 7F), all of which were coupled to phycoerythrin (n=2).

The paragraph at page 8, lines 15 through 24 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

[Figure 10 is] Figures 10A-10F are a series of histograms illustrating that a subset of CD4 lymphocytes and thymocytes chemotax to TECK. CD4⁺ lymphocytes (Figure 10F), CD8⁺ lymphocytes (Figure 10B), CD56⁺ NK cells (Figure 10D) and CD14⁺ monocytes (Figure 10A) were isolated from mononuclear cells using the appropriate Miltenyi Beads. Neutrophils (Figure 10E) were isolated by dextran precipitation followed by Ficoll and eosinophils (Figure 10C) separated from neutrophils by depletion with anti-CD16 Miltenyi Beads. Uncoated 3 μ m Costar plates were used to assess chemotaxis with these leukocyte subsets, with the exception of eosinophils and neutrophils, for which ECV304 monolayers were grown over the inserts before the assay. In each

case, TECK was tested in a dose response fashion between 1 nM and 220 nM. Chemokines known to act on the leukocyte subsets were used as positive controls (n=2).

The paragraph bridging pages 14 and 15 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

As described herein, an antibody designated "mAb 3C3" that binds human GPR-9-6 has been produced. mAb 3C3 can be produced by murine hybridoma 3C3, also referred to as murine hybridoma LS129-3C3-E3-1 which was deposited on March 4, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A., now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 01239, at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, U.S.A., under Accession No. [] HB-12653. In another embodiment, the anti-GPR-9-6 antibody of the invention is mAb 3C3 or an antigen-binding fragment thereof. In another embodiment, the binding of the antibody or antigen-binding fragment to mammalian (e.g., human) GPR-9-6 can be inhibited by mAb 3C3. Such inhibition can be the result of competition for the same or similar epitope, steric interference or due to a change in the conformation of GPR-9-6 that is induced upon antibody binding to the receptor. In still another embodiment, the antibody or antigen-binding fragment of the invention has the same or similar epitopic specificity as mAb 3C3. Antibodies with an epitopic specificity which is the same as or similar to that of mAb 3C3 can be identified by a variety of suitable methods. For example, an antibody with the same or similar epitopic specificity as mAb 3C3 can be identified based upon the ability to compete with mAb 3C3 for binding to mammalian GPR-9-6. In another example, the binding of mAb 3C3 and the binding of an antibody with the same or similar epitopic specificity to mammalian GPR-9-6 can be inhibited by a single peptide (e.g., natural peptide, synthetic peptide). The peptide can comprise nine to about fifty amino acids. Preferably, the peptide comprises nine to about twenty-six amino acids. In still another example, an antibody with the same or similar epitopic specificity as mAb 3C3 can be identified using chimeric receptors (see e.g., Rucker *et al.*, *Cell* 87:437-446 (1996)).

The paragraph at page 15, lines 22 through 28 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

The invention also relates to a bispecific antibody, or functional fragment thereof (e.g., F(ab')₂), which binds to a mammalian GPR-9-6 and at least one other antigen. In a particular embodiment, the bispecific antibody, or functional fragment thereof has the same or similar epitopic specificity as mAb 3C3 and at least one other antibody (see, e.g., U.S. Patent No. 5,141,736 (Iwasa *et al.*), U.S. Patent Nos. 4,444,878, 5,292,668, 5,523,210 (all to Paulus *et al.*) and U.S. Patent No. 5,496,549 (Yamazaki *et al.*)).

The paragraph bridging pages 19 and 20 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

In one embodiment, a functional variant of mammalian GPR-9-6 (e.g., a ligand binding variant) shares at least about 80% amino acid sequence similarity with said mammalian GPR-9-6, preferably at least about 90% amino acid sequence similarity, and more preferably at least about 95% amino acid sequence similarity with said mammalian GPR-9-6. In another embodiment, a functional fusion protein comprises a first moiety which shares at least about 85% sequence similarity with a mammalian GPR-9-6, preferably at least about 90% sequence similarity, and more preferably at least about 95% sequence similarity with a mammalian GPR-9-6 (e.g., a human GPR9-6 (e.g., SEQ ID NO:2)). In another embodiment, a functional mammalian GPR-9-6 protein or functional variant of a mammalian GPR-9-6 protein shares at least about 80% amino acid sequence similarity, preferably at least about 90% amino acid sequence similarity, and more preferably at least about 95% amino acid sequence similarity with a naturally occurring human GPR-9-6 (e.g., SEQ ID NO:2). Amino acid sequence similarity can be determined using a suitable sequence alignment algorithm, such as the [Lasergene] LASERGENE system (sequence assembly and alignment software; DNASTAR, Inc., Madison, WI), using the Clustal method with the PAM 250 residue weight table, a gap penalty of 10, a gap length penalty of 10 and default parameters (pairwise alignment parameters: ktuple = 1, gap penalty = 3, window = 4 and diagonals saved = 5). In another embodiment, a functional variant is

encoded by a nucleic acid sequence which is different from the naturally-occurring nucleic acid sequence, but which, due to the degeneracy of the genetic code, encodes mammalian GPR-9-6 or a portion thereof.

The paragraph bridging pages 20 and 21 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

A composition comprising a mammalian GPR-9-6 or functional variant thereof can be used in a binding assay to detect and/or identify agents that can bind to the receptor. Compositions suitable for use in a binding assay include, for example, cells which naturally express a mammalian GPR-9-6 or functional variant thereof (e.g., thymocytes, GPR-9-6⁺ CLA^{-ve} $\alpha 4\beta 7^{hi}$ CD4⁺ memory lymphocytes, cell lines (e.g., MOLT-4 (ATCC Accession No. CRL-1582), MOLT-13 (M. Brenner, Brigham and [Womans] Women's Hospital, Boston, MA)) and recombinant cells comprising an exogenous nucleic acid sequence which encodes a mammalian GPR-9-6 or functional variant thereof. Compositions suitable for use in a binding assay also include, membrane preparations which comprise a mammalian GPR-9-6 or functional variant thereof. Such membrane preparations can contain natural (e.g., plasma membrane) or synthetic membranes. Preferably, the membrane preparation is a membrane fraction of a cell that expresses a mammalian GPR-9-6 or a functional variant thereof.

The paragraph at page 25, lines 3 through 15 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

An agent which binds to a mammalian GPR-9-6 can also be assessed by monitoring cellular responses induced by active receptor, using suitable cells which express a mammalian GPR-9-6 or a functional variant thereof. For instance, exocytosis (e.g., degranulation of cells leading to release of one or more enzymes or other granule components, such as esterases (e.g., serine esterases), perforin, and/or granzymes), inflammatory mediator release (such as release of bioactive lipids such as leukotrienes (e.g., leukotriene C₄)), and respiratory burst, can be monitored by methods known in the

art or other suitable methods (see e.g., Taub, D.D. *et al.*, *J. Immunol.*, 155: 3877-3888 (1995), regarding assays for release of granule-derived serine esterases; Loetscher *et al.*, *J. Immunol.*, 156: 322-327 (1996), regarding assays for enzyme and granzyme release; Rot, A. *et al.*, *J. Exp. Med.*, 176: 1489-1495 (1992) regarding respiratory burst; Bischoff, S.C. *et al.*, *Eur. J. Immunol.*, 23: 761-767 (1993) and [Baggiolini] Baggiolini, M. and C.A. Dahinden, *Immunology Today*, 15: 127-133 (1994)).

The paragraph bridging pages 27 and 28 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

In vivo models of inflammation are available which can be used to assess the efficacy of antibodies and antigen-binding fragments of the invention as well as agents identified by the methods described herein as *in vivo* as therapeutics. For example, leukocyte infiltration upon intradermal injection of a chemokine and an antibody or fragment thereof reactive with mammalian GPR-9-6 into a suitable animal, such as rabbit, mouse, rat, guinea pig or primate (e.g., rhesus macaque) can be monitored (see e.g., Van Damme, J. *et al.*, *J. Exp. Med.*, 176: 59-65 (1992); Zachariae, C.O.C. *et al.*, *J. Exp. Med.* 171: 2177-2182 (1990); Jose, P.J. *et al.*, *J. Exp. Med.* 179: 881-887 (1994)). In one embodiment, skin biopsies are assessed histologically for infiltration of leukocytes (e.g., GPR-9-6⁺ T cells). In another embodiment, labeled cells (e.g., stably transfected cells expressing a mammalian GPR-9-6, labeled with ¹¹¹In for example) capable of chemotaxis and extravasation are administered to the animal. For example, an antibody or agent to be assessed which binds a mammalian GPR-9-6 can be administered, either before, simultaneously with or after a GPR-9-6 ligand or agonist (e.g., TECK) is administered to the test animal. A decrease of the extent of infiltration in the presence of antibody or agent as compared with the extent of infiltration in the absence of said antibody or agent is indicative of inhibition.

The paragraph bridging pages 41 and 42 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Plasmid DNA was isolated using QIAGEN-tips as recommended by the manufacturer (QIAGEN Inc., Chatsworth, CA). DNA ligations, restriction endonuclease digestions, and gel electrophoresis were performed as described previously (Sambrook, J., *et al.*, *Molecular Cloning: A Laboratory Manual 2nd ed.*, Cold Spring Harbor Laboratory Press, (Cold Spring Harbor, NY) (1989)). DNA purification through agarose gel extraction was performed using the QIAEXII Gel Extraction Kit as recommended by the manufacturer (QIAGEN Inc., Chatsworth, CA). Plasmid DNA was introduced into *E. coli* by chemical transformation (GIBCO, Inc.). Enzymes were purchased from New England Biolabs, Inc. (Beverly, MA), GIBCO Bethesda Research Laboratories, Inc. (Gaithersburg, MD), or from Boehringer Mannheim, Inc. (Germany). RNA was isolated from frozen tissues or cells using either the standard guanidinium isothiocyanate method (Sambrook, J., *et al.*, *Molecular Cloning: A Laboratory Manual 2nd ed.*, Cold Spring Harbor Laboratory Press, (Cold Spring Harbor, NY) (1989)) or the RNeasy kit as recommended (QIAGEN Inc., Chatsworth, CA). DNA sequencing was performed by Sequi-Net (Colorado State University) using the FS DyeDeoxy Terminator cycle sequencing kit and a model 377 DNA sequencer (Perkin Elmer Applied Biosystems, Foster City, CA). Sequences were analyzed using SeqMan (DNASTAR, Inc., Madison, WI).

The paragraph bridging pages 45 and 46 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

In initial two color studies of peripheral blood, GPR-9-6 was found to be expressed on a small subset (2-4%) of CD4 lymphocytes as well as on a very small subset of CD8 lymphocytes [(Figure 3)], while B lymphocytes expressed low and heterogeneous levels of GPR-9-6 (Figures 3A-3C). Monocytes, basophils, eosinophils, neutrophils and NK cells did not express GPR-9-6 under the conditions used ([Figure 3] Figures 3D-3I). GPR-9-6 was expressed on a large subset of thymocytes expressing all levels of TcR, although a small subset of TcR^{high}GPR-9-6^{-ve} thymocytes was evident. In three-color experiments, GPR-9-6 was found on the majority of CD4, CD8 and CD4^{+ve}CD8^{+ve} thymocytes and on approximately 50% of immature CD4^{-ve}CD8^{-ve} thymocytes (data not shown). No expression of GPR-9-6 was seen on either immature or mature dendritic cells

(Figure 4D). However, as expected, immature dendritic cells expressed CCR5, which was down-regulated on LPS activation, while CD83 and CD86 were up-regulated (Figures 4A-4C). In examining a large panel of cell lines GPR-9-6 was found on several T cell lines (Table 1). Umbilical CD4+ lymphocytes did not express GPR-9-6 (Figure 4E) and chronic activation of these cells in the presence of IL-12 or IL-4 to generate T_H1 or T_H2 lymphocytes failed to induce the expression of GPR-9-6 (Figure 4H). However, as expected, CXCR3 were clearly up-regulated on T_H1 lymphocytes (Figure 4F), while $\alpha 4\beta 7$, an integrin utilized in lymphocyte trafficking to mucosal sites, was up-regulated on both T_H1 and T_H2 lymphocytes (Figure 4G).

The paragraph at page 46, lines 5 through 10 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Expression of GPR-9-6 on CD4 lymphocytes and B lymphocytes was measured over time, and was found to be relatively constant (Figure 5A). However, activation of T lymphocytes with anti-CD3 mAb resulted in transient down-regulation of GPR-9-6 over 2 days, with expression recovering after 10 days of culture in IL-2 (Figure 5B). Chemokine receptors CCR6 and CCR5 [shown] showed similar changes in expression upon T lymphocyte activation (Figure 5C).

The paragraph at page 46, lines 14 through 24 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

The small subset of CD4 lymphocytes that express GPR-9-6 were examined in more detail by three-color staining ([Figure 6] Figures 6A-6F). The CD4 lymphocytes that express GPR-9-6 were mainly of memory phenotype, and those cells that expressed the highest levels of GPR-9-6 were all of memory phenotype. Interestingly, memory CLA^{+ve} CD4 lymphocytes, which traffic to skin, did not express GPR-9-6. In contrast, a subset of memory $\alpha 4\beta 7^{\text{high}}$ CD4 lymphocytes, which traffic to mucosal sites, clearly expressed GPR-9-6. The subset of memory CD4 lymphocytes defined by expression of $\alpha E\beta 7$ were also clearly subdivided into GPR-9-6 positive and negative subsets. GPR-

9-6^{high} CD4 lymphocytes did not express CD62L, a homing receptor which is involved in trafficking to peripheral lymph nodes, while a small subset of GPR-9-6^{dull}CD62L^{+ve} lymphocytes was evident.

The paragraph bridging pages 46 and 47 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

GPR-9-6^{+ve} CD4 lymphocytes were also examined for co-expression of other chemokine receptors known to be expressed on CD4 lymphocytes ([Figure 7] Figures 7A-7F). While GPR-9-6 was clearly found on both positive and negative subsets of CCR5, CCR6, CXCR3 and CXCR5, CD4 lymphocyte expression of CCR2 and GPR-9-6 was mutually exclusive.

The paragraph at page 49, lines 1 through 10 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Leukocyte subsets were also tested ([Figure 10] Figures 10A-10F) to determine if they chemotaxed to TECK. As observed in the mouse, neutrophils, monocytes, eosinophils, CD8 and NK cells did not chemotax to TECK, but did chemotax to other chemokines. However, TECK was chemotactic for a minor subset of CD4 lymphocytes. As murine TECK induces thymocyte chemotaxis, chemotaxis of human thymocytes to TECK and SDF1 α , both of which mediate thymocyte chemotaxis (data not shown) was examined. Anti-GPR-9-6 mAb 3C3 blocked thymocyte and CD4 lymphocyte chemotaxis to TECK. The anti-GPR-9-6 mAb 3C3 had no effect on TARC-induced chemotaxis of CD4 lymphocytes, indicating that the effect is specific (Figures 11A-11C). These results indicate that GPR-9-6 is the major physiological receptor for TECK.

The paragraph at page 50, lines 7 through 20 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Several different adhesion molecules are involved in trafficking of lymphocyte subsets to distinct physiologic location, such as peripheral lymph node ([Gallatin] Gallatin, W.M., *et al.*,

Nature, 304:30-34 (1983)), [Peyers] Peyer's Patches (Hamman, A., *et al.*, *J. Immunol.*, 152:3282-3292 (1994); Andrew, D.P., *et al.*, *Eur. J. Immunol.*, 26:897-905 (1996)) and inflammatory sites (Frenette, P.S., *et al.*, *Cell*, 84:563-574 (1996); Tietz, W.Y., *et al.*, *J. Immunol.*, 161(2):963-970 (1998); Picker, L.J., *et al.*, *J. Immunol.*, 145:3247-3255 (1990)). It is thought that specific chemokine receptors expressed on these lymphocyte subsets may interact with chemokines expressed in the areas mediating leukocyte activation, arrest, and transendothelial migration. It is therefore possible that CD4 subsets defined by the expression of certain adhesion molecules, may also express known, orphan or as yet undiscovered chemokine receptors that are important for trafficking of the lymphocytes into these sites. The work described herein relates to one such chemokine receptor that may be involved in the selective trafficking of memory CD4 and CD8 lymphocyte subsets to mucosal sites.

The paragraph bridging pages 52 and 53 is shown below, marked up by way of bracketing and underlining, to show the changes relative to the previous version of the paragraph.

Out of all the chemokines tested only TECK (Vicari, A.P., *et al.*, *Immunity*, 7(2):291-301 (1997)) acted as a chemoattractant for GPR-9-6/L1.2 transfectants, with 150 nM resulting in optimal chemotaxis. This falls into the range of 1nM-1 μ M for which other leukocyte chemokines are active. However, as we are using TECK that was generated by peptide synthesis, we cannot be sure that either post-translational modifications or further cleavage of TECK by factors outside the cell *in vivo* do not generate more active fragments, as is the case for CKB8 (Macphee, C.H., *et al.*, *J. Immunol.* 161:6273-6279 (1998)). TECK did not act as a chemoattractant for CCR1, CCR2, CCR4, CCR5, CCR6, CCR7, CCR9 and CXCR1, CXCR2, CXCR3, CXCR4 and CXCR5 L1.2 transfectants. However, some weak activity of TECK on CCR3/L1.2 transfectants which was approximately 20% of the chemotactic activity observed with eotaxin-1 was detected. This activity was blocked by anti-CCR3 mAbs, though TECK did not act as a chemoattractant for eosinophils. Therefore, TECK is probably not a physiological chemokine for the CCR3 receptor. This result is not unprecedented, as in previous studies MIP-1 α has been shown to act as a chemoattractant for CCR4/HEK293 transfectants (Power, C.A., *et al.*, *J. Biol. Chem.*, 270:19495-19500 (1995)), but not CCR4/L1.2

transfectants (Imai, T.M., *et al.*, *J. Biol. Chem.*, 272:15036-15042 (1997)). In further experiments, only the T cell lines that express GPR-9-6 were found to chemotax to TECK, while among primary cells TECK was chemotactic for only a small subset of CD4 lymphocytes. Presumably, these cells represent the small subset of CD4 lymphocytes that express GPR-9-6, as the chemotaxis was blocked by anti-GPR-9-6 mAb 3C3. Additionally, only $\alpha 4\beta 7^{+ve}$ memory CD4 and CD8 lymphocytes chemotax to TECK, which would be the subset predicted to express GPR-9-6. TECK was originally described as a chemokine produced by thymic dendritic cell, whose expression is restricted to thymus and small intestine (Vicari, A.P., *et al.*, *Immunity*, 7(2):291-301 (1997)). Our Northern data confirms this observation and shows that the receptor for TECK, GPR-9-6, is also expressed at these sites. The expression of both chemokine receptor GPR-9-6 and its ligand TECK in small intestine and thymus predict a role for GPR-9-6 and TECK in T cell development and mucosal immunology.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Claims 1-24 and 29-47 have been canceled and Claims 50-96 have been added

25. (Amended) A method of detecting or identifying an inhibitor of a mammalian GPR-9-6 receptor comprising:
 - a) combining an agent to be tested, a ligand or promoter of [said] mammalian GPR-9-6 and a cell expressing [said] mammalian GPR-9-6 under conditions suitable for detecting a ligand- or promoter-induced response; and
 - b) determining the ability of the test [compound] agent to inhibit said ligand- or promoter-induced response,
wherein inhibition of [a] said ligand- or promoter-induced response by the agent is indicative that the agent is an inhibitor.
28. (Amended) The method of Claim 25 wherein said ligand- or promoter-induced response is chemotaxis [or Ca^{2+} flux].